PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
295.009WO1		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US 96/10211	07/06/1996	07/06/1995
International Patent Classification (IPC) or national classification and IPC		
A61K31/135		
Applicant		
NEORX CORP. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of 26 sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/01/1997	26.08.97

Name and mailing address of the IPEA/

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I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

[ ] the international application as originally filed.

[x] the description, pages 1-105, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____,

[x] the claims, Nos. 1-22, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 23-152, filed with the letter of 21.07.97,
Nos. _____, filed with the letter of _____,

[ ] the drawings, sheets/fig 1-4, as originally filed,
sheets/fig _____, filed with the demand,
sheets/fig _____, filed with the letter of _____,
sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[ ] the description, pages _____.
[ ] the claims, Nos. _____.
[ ] the drawings, sheets/fig _____.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

[ ] the entire international application,

[x] claims Nos. 2,21-23,31,42-51,61-124,126-132,138,141-

because:

[x] the said international application, or the said claims Nos. 122, 123 relate to the following subject matter which does not require an international preliminary examination (specify):

1). See section VIII and Rule 70.2(c) PCT.

[x] the description, claims or drawings (indicate particular elements below) or said claims Nos. see above are so unclear that no meaningful opinion could be formed (specify):

See section VIII

[ ] the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

[ ] no international search report has been established for said claims Nos. _____.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

[ ] restricted the claims.
[ ] paid additional fees.
[ ] paid additional fees under protest.
[ ] neither restricted nor paid additional fees.

2. [x] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

[ ] complied with.
[x] not complied with for the following reasons:

See section VIII

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

[x] all parts.*
[ ] the parts relating to claims Nos. _____.

* All parts not excluded in section III

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1,3-20,24-30,33,38-41,53,54,125,133-137,139	YES
	Claims 32,34-37,52,55-60	NO
Inventive Step (IS)	Claims	YES
	Claims 1,3-20,24-30,33,38-41,53,54,125,133-137,139	NO
Industrial Applicability (IA)	Claims 1,2-20,32-41,52-60,125,133-137,139	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

1). Document D1 (WO 94/26303) discloses in claim 22 a method of determining TGF-β in vitro, identical to that of the present claim 32. D1 page 50 line 33 discloses the detction of both TGF-β1 and β3. This claim therefore does not meet the requirements of Article 33(2) PCT.

2). The features of claims 35 to 37 would appear to be repetition of D1 claims 23 to 26 (Art 33(2) PCT.

3). The features of remaining test method claims 33, 34, 38-41 would appear to be obvious development of D1 under Article 33(3) PCT. There appears to be nothing in the present description which shows these features to have technical effects which could not have been predicted from D1.

4). The method of D1 claim 22 also appear to be identical to that of the kit claim 52 (S e also D1 examples 1 to 3). Claims 52, 55, 56, 57, 59, and 60 therefore would not appear to be novel under Article 33(2) PCT.
Claims 53, 54 would appear to be obvious developments of the method of D1 under Article 33(3) PCT. There appears to be nothing in the description which shows that these developments have lead to a result which could not have been predicted.

5). D1 discloses the use of Tamoxifen to treat atherosclosrosis. (see claims 1 ,2 and 11). The present claim 1 is novel over this disclosure as R3 cannot be ethyl when R4, R5 and R6 are hydrogen. It is not however apparent from the description how the use of analogues of Tamoxifen shows and effect which could not have been predicted from D1. Example 6 on page 69 lines 10 to 14 shows that both TMX and IDX inhibited cell growth "to a similar extent".

For the skilled man the use of known analogues would appear to be obvious, and he would expect "similar" results. No inventive step can therefore be recognised for claims 1, 2-20, 24-30, 125, 133-137 and 139 under Article 33(3) PCT.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1). The application does not meet the requirements of Rule 13 PCT as there appears to be two separate "inventions". The provision of a new method of treatment, claim 1 and a analytical method claim 32 appear to address different problems, once the underlying mechanism is known. (See D1).

2). Rule 6 PCT states that the number of claims shall be reasonable. There appears to be no reason whatsoever for the proliferation of independent claims. There appears for example to be 12 (originally 11) independent method of treatment claims. The actual intended scope of the patent is therefore grossly unclear to the reader. The examination has therefore been restricted to the first independent claim in each category.

4). As tamoxifen is not a part of formula I there appears to be no relevance for numerous examples detailing results with tamoxifen alone. These could only be of relevance if they compared tamoxifen with a compound which actually is a part of the "invention" (Rule 9.1 (iv) PCT).

5). For the assessment of the present claims 1-24 and 65-128 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for th manufacture of a medicament for a n w medical treat-

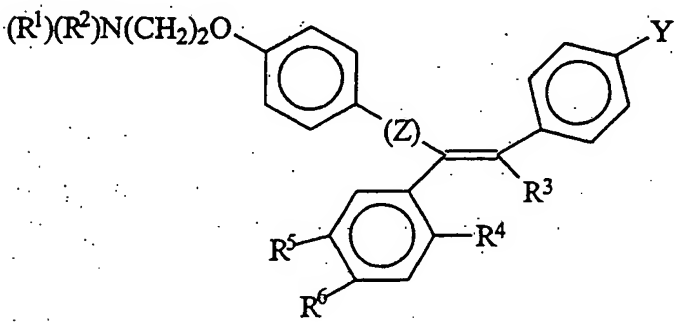
ment.

6). There appears to be no basis for the new claims 138, 140, and 141 (Art 34.2(b) PCT.

23. The method of claim 21 wherein the compound of formula (I) is idoxifene or a pharmaceutically acceptable salt thereof.

24. The method of claim 1, 2 or 21 wherein the compound indirectly or directly increases the level of active TGF-beta.

25. A kit comprising, separately packaged, a catheter adapted for the local delivery of a therapeutic agent to a site in the lumen of a mammalian vessel and a unit dosage of a therapeutic agent of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H, R^5 is I, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof, wherein the unit dosage is effective to inhibit pathological activity of the smooth muscle cells at said site.

26. The kit of claim 25 wherein the catheter is adapted to deliver the unit dosage form to an arterial lesion.

AMENDED SHEET

27. The kit of claim 25 wherein the catheter is adapted to deliver the unit dosage to a vessel site which has been subjected to coronary angioplasty.

28. The kit of claim 25 wherein the therapeutic agent of formula (I) is idoxifene or a pharmaceutically acceptable salt thereof.

29. The kit of claim 25 wherein the therapeutic agent of formula (I) is toremifene or a pharmaceutically acceptable salt thereof.

30. The kit of claim 25 wherein the therapeutic agent of formula (I) indirectly or directly increases the level of active TGF-beta.

31. A kit comprising, separately packaged, a catheter adapted for the local delivery of a therapeutic agent to a site in the lumen of a mammalian vessel and a unit dosage of droloxifene and pharmaceutically acceptable salts thereof, wherein the unit dosage is effective to inhibit pathological activity of the smooth muscle cells at said site.

32. A method for determining TGF-beta-1 or TGF-beta-3 *in vitro*, said method comprising:

(a) contacting a mammalian blood-derived sample with a capture moiety, to form a capture complex comprising said capture moiety and TGF-beta-1 or TGF-beta-3;

(b) contacting the capture complex with a detection moiety which binds TGF-beta-1 or TGF-beta-3 and which comprises a detectable label, or a site which binds a detectable label, to form a detectable complex; and

(c) detecting the presence of the detectable complex, so as to determine the presence of TGF-beta-1 or TGF-beta-3 in said sample, thereby identifying a mammal at risk for atherosclerosis or the effect of administering to a mammal a therapeutic agent

which increases the level of TGF-beta-1 or TGF-beta-3 in said mammal.

33. The method of claim 32 wherein the capture moiety is immobilized on a solid substrate.

34. The method of claim 32 wherein the capture moiety is a solution phase capture moiety.

35. The method of claim 32 wherein the capture moiety or the detection moiety is capable of binding both latent and active TGF-beta-1 or TGF-beta-3.

36. The method of claim 32 wherein the capture moiety is a first antibody and the detection moiety is a second antibody.

37. The method of claim 32 wherein the capture moiety comprises a TGF-beta type II receptor extracellular domain and the detection moiety is an antibody.

38. The method of claim 32 wherein the presence of the detectable complex is detected by reacting the detectable complex with an antibody comprising a detectable label, which binds to said detectable complex, and determining the presence of the label.

39. The method of claim 32 wherein the capture or the detection moiety comprises a fusion protein comprising the TGF-beta type II extracellular domain.

40. The method of claim 39 wherein the TGF-beta type II extracellular domain has a methionine residue at position 5.

41. The method of claim 32 wherein the moiety that binds active but not latent TGF-beta-1 or TGF-beta-3 is a fusion protein comprising the TGF-beta Type II extracellular domain.

42. A method for determining TGF-beta-1 or TGF-beta-3 *in vitro*, consisting essentially of:

(a) contacting a blood-derived sample from an individual with a capture moiety which binds TGF-beta-1 or TGF-beta-3, to form a capture complex comprising said capture moiety and TGF-beta-1 or TGF-beta-3;

(b) combining the capture complex with a detection moiety which binds TGF-beta-1 or TGF-beta-3 and which has a detectable label or a site which binds a detectable label, to form a detectable complex; and

(c) determining the presence of a detectable label in the detectable complex, so as to determine the presence of TGF-beta-1 or TGF-beta-3 in the sample.

43. The method of claim 42 wherein the detection moiety is an antibody.

44. The method of claim 42 wherein the capture moiety is an antibody.

45. The method of claim 42 wherein the moiety that binds active but not latent TGF-beta-1 or TGF-beta-3 comprises a fusion protein comprising the TGF-beta type II receptor extracellular domain.

46. The method of claim 45 wherein the TGF-beta type II receptor extracellular domain has a methionine residue at position 5.

47. The method of claim 39 or 41 wherein fusion protein is a prokaryotic fusion protein.

48. The method of claim 32 or 42 wherein the blood-derived sample is serum or plasma.

49. The method of claim 32 wherein either or both the capture moiety or the detection moiety bind active TGF-beta-1 or TGF-beta-3 but not latent TGF-beta-1 or TGF-beta.

50. The method of claim 49 wherein the presence of active TGF-beta-1 or TGF-beta-3 identifies a mammal at risk for atherosclerosis or monitors the effect of administering to a mammal a therapeutic agent which increases the level of TGF-beta-1 or TGF-beta-3 in said mammal.

51. The method of claim 42 wherein either or both the capture moiety or the detection moiety bind active TGF-beta-1 or TGF-beta-3 but not latent TGF-beta-1 or TGF-beta.

52. A test kit for determining the level of TGF-beta-1 or TGF-beta-3 in a physiological sample obtained from a mammal *in vitro*, comprising packaging material enclosing, separately packaged, (a) a capture moiety capable of binding TGF-beta-1 or TGF-beta-3; (b) a detection moiety capable of binding TGF-beta-1 or TGF-beta-3, which moiety comprises a detectable label or a binding site for a detectable label; and (c) instruction means directing the user to correlate the level of TGF-beta-1 or TGF-beta-3 in the sample with the risk to said mammal of atherosclerosis or with the effect of the administration of a therapeutic agent which increases the level of TGF-beta-1 or TGF-beta-3 in said mammal.

53. The test kit of claim 52 wherein said capture moiety is immobilized on a solid substrate.

54. The test kit of claim 52 wherein said capture moiety is present in solution.

55. The test kit of claim 52 wherein the capture moiety is a first antibody.

56. The test kit of claim 52 wherein the detection moiety is a second antibody.

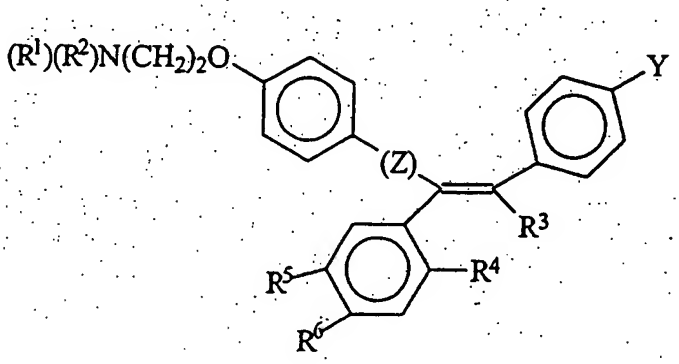
57. The test kit of claim 52 wherein the capture moiety comprises a TGF-beta type II receptor extracellular domain.

58. The test kit of claim 57 wherein the TGF-beta type II receptor extracellular domain is derived from a bacterial expression system.

59. The test kit of claim 52 wherein the detection moiety is an antibody.

60. The test kit of claims 56 or 59 further comprising, separately packaged, an antibody which binds to said detection moiety, which comprises a detectable label.

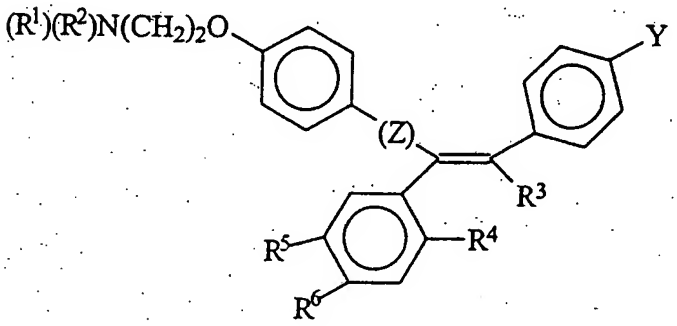
61. A therapeutic method comprising inhibiting smooth muscle cell (SMC) proliferation associated with procedural vascular trauma comprising the administration to a mammal subjected to said procedure, an effective cytostatic SMC proliferation inhibitory amount of a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H, R^5 is I, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof.

62. A therapeutic method comprising inhibiting vascular smooth muscle cell proliferation associated with procedural vascular trauma comprising administration to a mammal subjected to said procedural trauma an effective antiproliferative amount of a compound of formula (I):

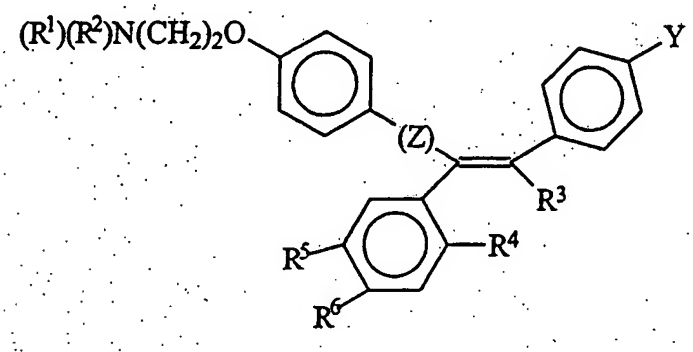


(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H, R^5 is I, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof.

63. A therapeutic method comprising inhibiting non-aortal vascular smooth muscle cell proliferation associated with procedural vascular trauma comprising administering to a mammal, such as a human, subjected to

said procedural vascular trauma an effective cytostatic antiproliferative amount of a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is -CH_2-CH_2- or -S-, R^5 is I, OH, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H; or a pharmaceutically acceptable salt thereof.

64. The method of claim 61 wherein the procedural vascular trauma is due to organ transplantation, vascular surgery, transcatheter vascular therapy, vascular grafting, placement of a vascular shunt or placement of an intravascular stent.

65. The method of claim 63 wherein the compound of formula (I) is tamoxifen or a pharmaceutically acceptable salt thereof.

66. The method of claim 61 or 62 wherein the compound of formula (I) is idoxifene, 4-iodotamoxifen, 3-iodotamoxifen, toremifene, or a pharmaceutically acceptable salt thereof.

67. The method of claim 61, 62 or 63 wherein the compound of formula (I) is idoxifene or a pharmaceutically acceptable salt thereof.

68. The method of claim 21, 61, 62 or 63 wherein the compound of formula (I) is toremifene or a pharmaceutically acceptable salt thereof.

69. The method of claim 61, 62 or 63 wherein the administration is to a human patient.

70. The method of claim 61, 62 or 63 wherein the administration is before, during or after said procedure.

71. The method of claim 61, 62 or 63 wherein the administration is in a series of spaced doses.

72. The method of claim 61, 62 or 63 wherein the administration is parenteral.

73. The method of claim 61, 62 or 63 wherein the administration is oral.

74. The method of claim 61, 62 or 63 wherein the administration is systemic.

75. The method of claim 61, 62 or 63 wherein the compound of formula (I) is administered via a sustained release dosage form.

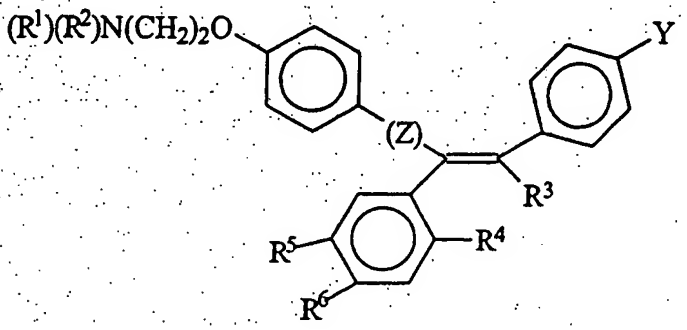
76. The method of claim 61, 62 or 63 wherein the administration is localized at the site of the vascular trauma.

77. The method of claim 61, 62 or 63 wherein the compound directly or indirectly increases the level of active TGF-beta.

78. The method of claim 63 wherein the compound of formula (I) is raloxifene, or a pharmaceutically acceptable salt thereof.

79. The method of claim 63 wherein the compound of formula (I) is droloxifene, or a pharmaceutically acceptable salt thereof.

80. A therapeutic method for preventing or treating a cardiovascular or vascular indication characterized by a decreased lumen diameter comprising administering to a mammal at risk of or afflicted with said cardiovascular indication, a cytostatic dose of a therapeutic agent, wherein the therapeutic agent is a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H, R^5 is I, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof.

81. The method of claim 80 wherein the cytostatic dose is effective to increase the level of TGF-beta so as to decrease lesion formation or development, inhibit lipid accumulation, increase plaque stability, maintain or increase vessel lumen diameter, or any combination thereof.

82. The method of claim 80 wherein the compound of formula (I) is idoxifene, 4-iodotamoxifen, 3-iodotamoxifen, toremifene, or a pharmaceutically acceptable salt thereof.

83. The method of claim 80 wherein the compound of formula (I) is idoxifene or a pharmaceutically acceptable salt thereof.

84. The method of claim 80 wherein the compound of formula (I) is toremifene or a pharmaceutically acceptable salt thereof.

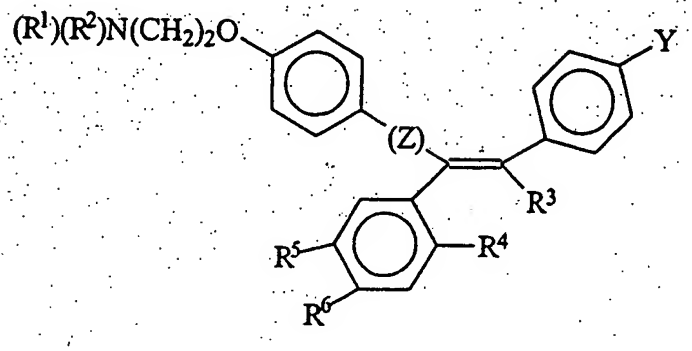
85. The method of claim 80 wherein the administration is systemic.

86. The method of claim 80 wherein the compound of formula (I) is administered via a sustained release dosage form.

87. The method of claim 80 wherein the administration is localized at the site of the vascular trauma.

88. The method of claim 80 wherein the compound directly or indirectly increases the level of active TGF-beta.

89. A therapeutic method of increasing the level of TGF-beta in a mammal in need thereof, comprising administering an effective amount of a compound of formula (I):



(I)

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wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is -CH_2-CH_2- or -S-, R^5 is I, OH, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof.

90. A method of treating diabetics at risk of, or afflicted with, vascular disease, comprising: administering an amount of tamoxifen or a structural analog thereof effective to indirectly or directly increase the level of active TGF-beta in said diabetic.

91. The method of claim 90 wherein the structural analog of tamoxifen is idoxifene, 4-iodotamoxifen, 3-iodotamoxifen, raloxifene, droloxifene, toremifene, or a pharmaceutically acceptable salt thereof.

92. The method of claim 90 wherein the structural analog of tamoxifen is idoxifene, 4-iodotamoxifen, 3-iodotamoxifen, toremifene, or a pharmaceutically acceptable salt thereof.

93. The method of claim 90 wherein the structural analog of tamoxifen is idoxifene, or a pharmaceutically acceptable salt thereof.

94. The method of claim 90 wherein the structural analog of tamoxifen is toremifene, or a pharmaceutically acceptable salt thereof.

95. The method of claim 89 or 90 wherein the increase in TGF-beta reduces or inhibits diabetic retinopathy.

96. The method of claim 89 wherein the mammal is diabetic.

97. The method of claim 96 wherein the diabetic has retinopathy.

98. The method of claim 89 wherein the compound indirectly or directly increases the level of active TGF-beta in vascular tissue.

99. The method of claim 1, 2, 21 or 89 wherein the compound is a TGF-beta production stimulator.

100. The method of claim 1, 2, 21 or 89 wherein the compound is a TGF-beta activator.

101. The method of claim 1, 2, 21 or 89 wherein the compound increases the production of TGF-beta mRNA.

102. The method of claim 1, 2, 21 or 89 wherein the compound increases the cleavage of the latent form of TGF-beta.

103. The method of claim 1, 2, 21 or 89 wherein the compound increases the bioavailability of TGF-beta.

104. The method of claim 89 wherein the compound is idoxifene or a pharmaceutically acceptable salt thereof.

105. The method of claim 89 wherein the compound is toremifene or a pharmaceutically acceptable salt thereof.

106. The method of claim 89 wherein the compound is droloxifene or a pharmaceutically acceptable salt thereof.

107. The method of claim 89 wherein the compound is tamoxifen or a pharmaceutically acceptable salt thereof.

108. The method of claim 1, 2, 21, 61, 62, 63, 80 or 89 wherein the compound forms cellular DNA adducts at level which is reduced relative to DNA adduct formation by tamoxifen.

109. The method of claim 1, 2, 21, 61, 62, 63, 80 or 89 wherein the compound has estrogenic activity which is reduced relative to the estrogenic activity of tamoxifen.

110. The method of claim 21, 61, 62, 63, 80 or 89 wherein the compound does not form cellular DNA adducts.

111. The method of claim 1, 2, 21, 61, 62, 63, 80 or 89 wherein the compound has no estrogenic activity.

112. A method of increasing the level of TGF-beta in a mammal in need thereof, comprising administering an effective amount of an agent that directly or indirectly elevates the level of active TGF-beta in said mammal, wherein the agent has reduced estrogenic activity relative to tamoxifen, reduced DNA adduct formation relative to tamoxifen, or any combination thereof.

113. The method of claim 112 wherein the agent is a structural analog of tamoxifen or a pharmaceutically acceptable salt thereof.

114. The method of claim 112 wherein the agent is idoxifene or a pharmaceutically acceptable salt thereof.

115. The method of claim 112 wherein the agent is toremifene or a pharmaceutically acceptable salt thereof.

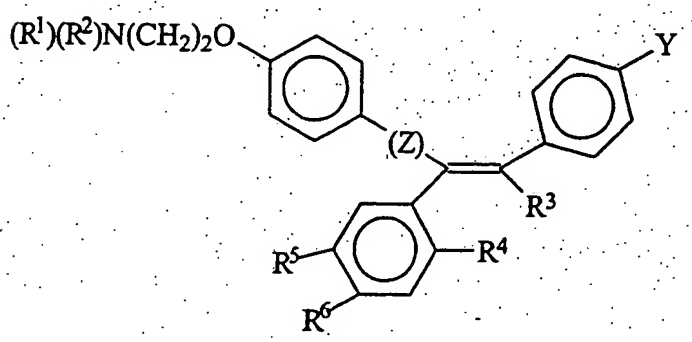
116. The method of claim 63 wherein the non-aortal smooth muscle cells which are inhibited are present in a non-coronary artery.

117. The method of claim 90 wherein the amount is effective to inhibit the proliferation of vascular tissue.

118. The method of claim 1, 2, 21, 61, 62, 63, 80, 89, 90 or 112 wherein the administration increases the level of latent TGF-beta relative to the level of latent TGF-beta prior to said administration.

119. The method of claim 1, 2, 21, 61, 62, 63, 80, 89, 90 or 112 wherein the administration increases the level of active TGF-beta relative to the level of active TGF-beta prior to said administration.

120. A therapeutic method for preventing or treating a cardiovascular or vascular indication characterized by a decreased lumen diameter comprising administering to a mammal at risk of or afflicted with said cardiovascular or vascular indication, a cytostatic dose of a therapeutic agent, wherein the therapeutic agent is a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is -CH_2-CH_2- or -S-, R^5 is I, OH, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5 and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof.

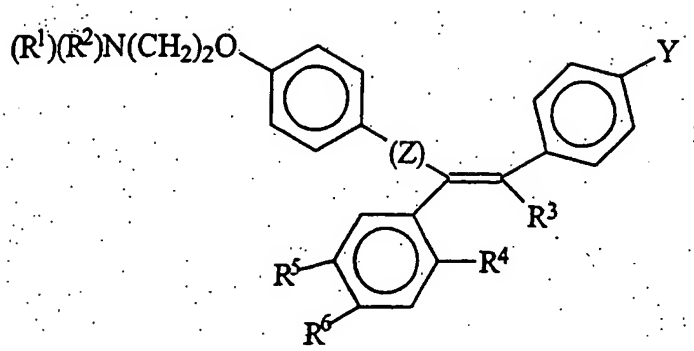
121. The method of claim 120 wherein the cytostatic dose is effective to increase the level of TGF-beta so as to decrease lesion formation or development, inhibit lipid accumulation, increase plaque stability, maintain or increase vessel lumen diameter, or any combination thereof.

122. The method of claim 120 wherein the compound of formula (I) is idoxifene, 4-iodotamoxifen, 3-iodotamoxifen, toremifene, or a pharmaceutically acceptable salt thereof.

123. The method of claim 120 wherein the administration is systemic.

124. The method of claim 120 wherein the compound of formula (I) is administered in a sustained release dosage form.

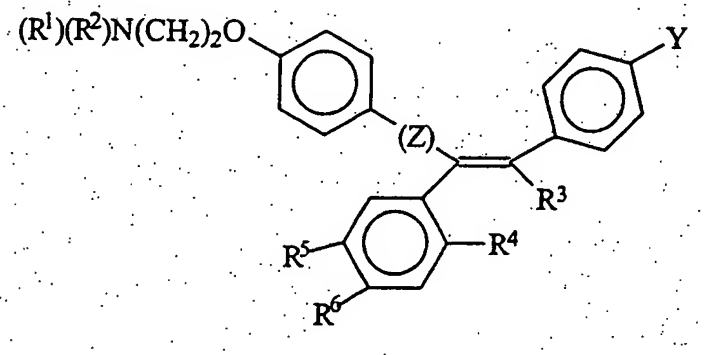
125. An intravascular stent comprising an amount of a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or $O(C_1-C_4)$alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is $-CH_2-CH_2-$ or -S-, R^5 is I, OH, $O(C_1-C_4)$alkyl or H and R^6 is I, $O(C_1-C_4)$alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

126. An intravascular stent comprising an amount of a compound of formula (I):

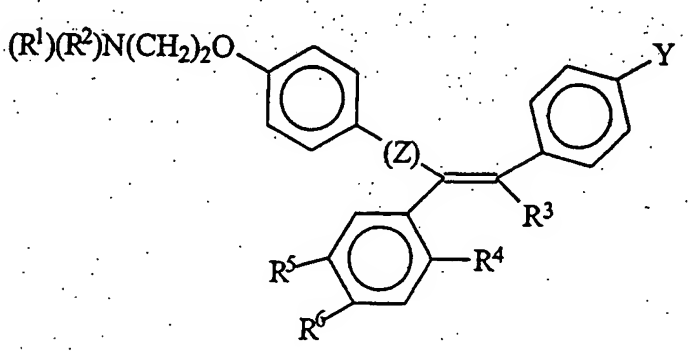


(I)

wherein Z is C=O or a covalent bond; Y is H or $O(C_1-C_4)$alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl, or together with N are a saturated

heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is $-CH_2-CH_2-$, R^5 is I, OH, $O(C_1-C_4)$alkyl or H and R^6 is I, $O(C_1-C_4)$alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

127. An intravascular stent comprising an amount of a compound of formula (I):

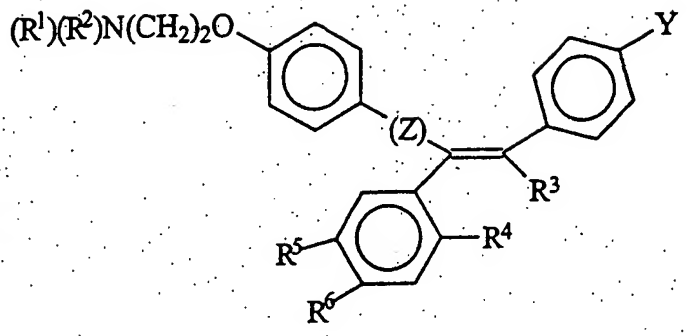


(I)

wherein Z is C=O or a covalent bond; Y is H or $O(C_1-C_4)$alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H; or together with R^3 is $-CH_2-CH_2-$ or -S-, R^5 is I, OH or $O(C_1-C_4)$alkyl and R^6 is I, $O(C_1-C_4)$alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

128. The stent of claim 122 or 123, wherein R^3 is not ethyl when R^4, R^5 and R^6 are H.

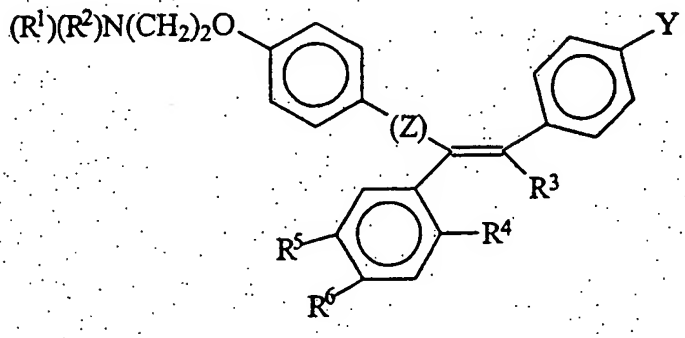
129. An intravascular stent comprising a cytostatic amount of a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is -CH_2-CH_2- or -S-, R^5 is I, OH, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

130. An intravascular stent comprising a cytostatic amount of a compound of formula (I):

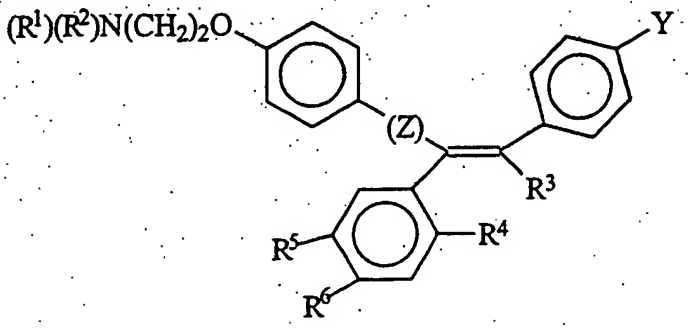


(I)

wherein Z is C=O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl, or together with N are a saturated

heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is $-CH_2-CH_2-$, R^5 is I, OH, $O(C_1-C_4)$alkyl or H and R^6 is I, $O(C_1-C_4)$alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

131. An intravascular stent comprising a cytostatic amount of a compound of formula (I):



(I)

wherein Z is C=O or a covalent bond; Y is H or $O(C_1-C_4)$alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is $-CH_2-CH_2-$ or -S-, R^5 is I, OH or $O(C_1-C_4)$alkyl and R^6 is I, $O(C_1-C_4)$alkyl or H; or a pharmaceutically acceptable salt thereof; effective to inhibit stenosis or reduce restenosis of a mammalian vessel following placement of the stent in said vessel.

132. The stent of claim 129 or 130, wherein R^3 is not ethyl when R^4, R^5 and R^6 are H.

133. The intravascular stent of any one of claims 125 to 132 that is adapted to maintain expanded vessel luminal area following angioplasty.

134. The intravascular stent of any one of claims 125 to 132 wherein the stent comprises a coating comprising the compound of formula (I).

135. The intravascular stent of any one of claims 122 to 129 wherein the compound of formula (I) is in a sustained release dosage form.

136. The intravascular stent of any one of claims 122 to 129 wherein the matrix of the stent comprises the compound of formula (I).

137. The intravascular stent of claim 134 wherein the coating is biodegradable.

138. The intravascular stent of claim 134 wherein the coating is porous or permeable to the inhibitor.

139. The therapeutic stent of any one of claims 125 to 132 wherein the matrix of the stent is formed from a porous or permeable non-biodegradable material.

140. The therapeutic stent of any one of claims 125 to 132 in which the intravascular stent comprises metal or plastic.

141. The therapeutic stent of any one of claims 125 to 132 wherein the matrix is formed from a biodegradable material.

142. A method for determining active TGF-beta levels, comprising:

(a) contacting a patient sample with a capture moiety that binds active TGF-beta-1 or active TGF-beta-1 and active TGF-beta-3,

to form a capture complex comprising said capture moiety and active TGF-beta-1 or active TGF-beta-1 and active TGF-beta-3, wherein the capture moiety comprises a TGF-beta extracellular domain comprising a signal peptide;

(b) contacting the capture complex with a detection moiety which comprises a detectable label, or a site which binds a detectable label, to form a detectable complex; and

(c) detecting the presence or amount of the detectable complex, so as to determine the presence or amount of active TGF-beta-1 or active TGF-beta-1 and active TGF-beta-3 in said sample.

143. A method for determining active TGF-beta levels, comprising:

(a) contacting a patient sample with a capture moiety that binds TGF-beta-1 or TGF-beta-1 and TGF-beta-3, to form a capture complex comprising said capture moiety and TGF-beta-1 or TGF-beta-1 and TGF-beta-3;

(b) contacting the capture complex with a detection moiety which comprises a detectable label, or a site which binds a detectable label, to form a detectable complex; and

(c) detecting the presence or amount of the detectable complex, so as to determine the presence or amount of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 in said sample, wherein the presence or amount of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 in said sample is correlated to the presence or amount of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 present *in vivo*.

144. A method for identifying a patient having, at risk of, a condition associated with a TGF-beta deficiency, comprising:

(a) contacting a patient sample with a capture moiety that binds TGF-beta-1 or TGF-beta-1 and TGF-beta-3, to form a capture complex

comprising said capture moiety and TGF-beta-1 or TGF-beta-1 and TGF-beta-3;

(b) contacting the capture complex with a detection moiety which comprises a detectable label, or a site which binds a detectable label, to form a detectable complex; and

(c) detecting the presence or amount of the detectable complex, so as to determine the presence or amount of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 in said sample, thereby identifying a patient having or at risk of a condition associated with a TGF-beta deficiency.

145. A method for monitoring a mammal that has received one or more administrations of a therapeutic agent to increase the level of TGF-beta, comprising:

(a) contacting a biological sample from said mammal with a capture moiety that binds TGF-beta-1 or TGF-beta-1 and TGF-beta-3, to form a capture complex comprising said capture moiety and TGF-beta-1 or TGF-beta-1 and TGF-beta-3;

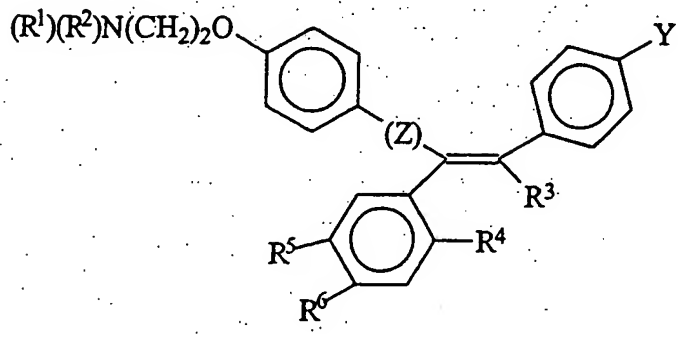
(b) contacting the capture complex with a detection moiety which comprises a detectable label, or a site which binds a detectable label, to form a detectable complex; and

(c) detecting the presence or amount of the detectable complex, so as to determine the presence or amount of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 in said sample, thereby identifying the effect of administering to a mammal a therapeutic agent which increases the level of TGF-beta-1 or TGF-beta-1 and TGF-beta-3 in said mammal.

146. A test kit for determining active TGF-beta-1 or TGF-beta-3 levels *in vitro*, comprising packaging material enclosing, separately packaged, (a) a capture moiety capable of binding TGF-beta-1 or TGF-beta-3, and (b) a

detection moiety capable of binding TGF-beta-1 or TGF-beta-3, which moiety comprises a detectable label or a binding site for a detectable label, wherein either or both the capture moiety or the detection moiety comprise a fusion protein comprising the TGF-beta type II receptor extracellular domain.

147. A therapeutic method comprising inhibiting vascular smooth muscle cell proliferation comprising administering to a mammal an effective cytostatic antiproliferative amount of a compound of formula (I):



(I)

wherein Z is C= O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H or together with R^3 is -CH_2-CH_2- or -S-, R^5 is I, OH, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H; or a pharmaceutically acceptable salt thereof, wherein the administration is by placement of a vascular shunt or intravascular stent comprising said compound.

148. The method of claim 150 wherein the compound is droloxifene, raloxifene, toremefine, tamoxifen, idoxifene, or a pharmaceutically acceptable salt thereof.

149. The method of claim 150 wherein the shunt or stent matrix is impregnated with the compound of formula (I).

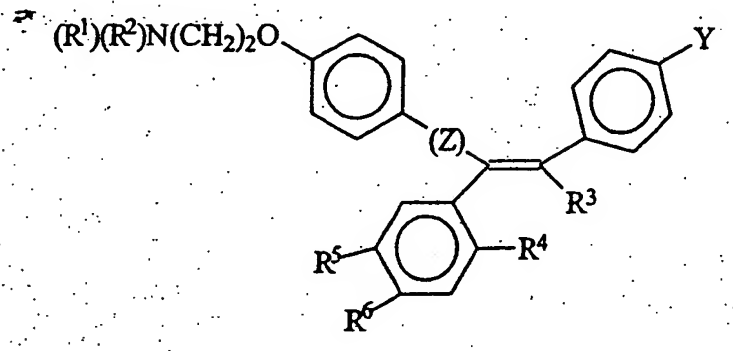
150. The method of claim 152 wherein the shunt or stent comprises a coating incorporating said compound of formula (I).

151. The method of claim 150 wherein the shunt or stent comprises a coating incorporating said compound of formula (I).

152. The method of claim 152, 153, or 154 wherein said matrix or said coating is biodegradable.

Abstract of the Invention

A method for treating or preventing cardiovascular pathologies by administering a compound of the formula (I):



(I)

wherein Z is C= O or a covalent bond; Y is H or O(C_1-C_4)alkyl, R^1 and R^2 are individually (C_1-C_4)alkyl or together with N are a saturated heterocyclic group, R^3 is ethyl or chloroethyl, R^4 is H, R^5 is I, O(C_1-C_4)alkyl or H and R^6 is I, O(C_1-C_4)alkyl or H with the proviso that when R^4, R^5, and R^6 are H, R^3 is not ethyl; or a pharmaceutically acceptable salt thereof, effective to elevate the level of TGF-beta to treat and/or prevent conditions such as atherosclerosis, thrombosis, myocardial infarction, and stroke is provided. Useful compounds include idoxifene, toremifene or salts thereof. Further provided is a method for identifying an agent that elevates the level of TGF-beta. Another embodiment of the invention is an assay or kit to determine TGF-beta *in vitro*. Also provided is a therapeutic method comprising inhibiting smooth muscle cell proliferation associated with procedural vascular trauma employing the administration of tamoxifen or structural analogs thereof, including compounds of formula (I).